Defining the Transfer Functions of the PCAD Model in North Atlantic Right Whales (*Eubalaena glacialis*) – Retrospective Analyses of Existing Data

Scott D. Kraus, Ph.D.
Vice President of Research
New England Aquarium
Central Wharf
Boston, MA 02110
phone: (617) 973-5457 fax: (617) 973-0242 email: skraus@neaq.org

Rosalind M. Rolland, D.V.M.
Senior Scientist
Research Department
New England Aquarium
Central Wharf
Boston, MA 02110
phone: (617) 973-6587 fax: (617) 973-0242 email: rrolland@neaq.org

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LONG-TERM GOALS

Anthropogenic noise has been shown to cause both behavioral and physiological changes in marine mammals, but the potential for long-term population level effects is not known. The NRC (2005) Population Consequences of Acoustic Disurbance (PCAD) model provides a framework to trace the consequences of acoustic disturbance through the life history of a marine mammal to its population status. In North Atlantic right whales (*Eubalaena glacialis*), extensive data on hormone levels, health and body condition, and individual life history exists. Our long term goal is to analyze the links between stress and thyroid hormones, visual assessments of health, and vital rates of right whales. This research supports the modeling efforts on PCAD transfer functions and develops a theoretical framework for field studies on acoustic disturbance for the North Atlantic right whale.

OBJECTIVES

The first objective is to test an alternative approach to elements of the PCAD model by: 1) substituting "behavior change" with direct measurements of physiological changes (using fecal hormone levels - Hunt et al. 2006; Rolland et al. 2005); 2) replacing "life function" with skin and body condition indices (Pettis et al. 2004), and 3) investigating the links between these parameters and right whale survival, reproduction and maturation (Figure 1). Analyses of retrospective data and new data on fecal thyroid hormones (in FY 2012) will set the stage to apply stress/thyroid hormone data and health indices to assess acoustic disturbance in right whales.

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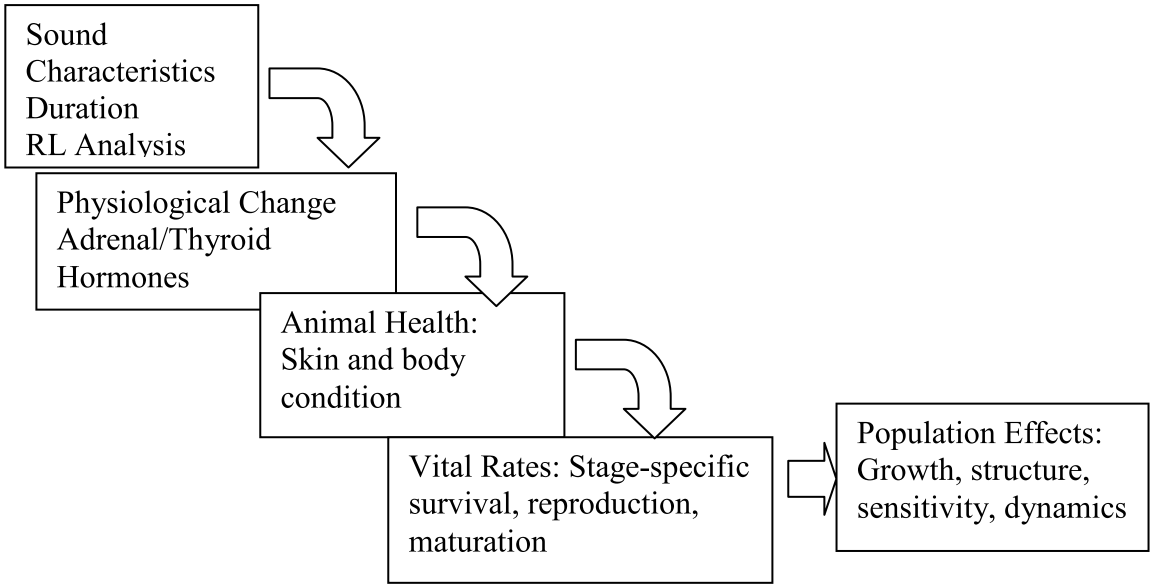

Figure 1. Right whales and acoustic disturbance – the proposed adaptation of the PCAD model. In this alternative framework, we substituted "behavioral changes" resulting from acoustic disturbance with "physiological changes" using adrenal and thryoid hormones, and the "life function changes" with "animal health changes" using visual assessment of body and skin condition.

A second objective of this project is to analyze both acoustic recordings and fecal stress hormone levels from the Bay of Fundy in 2001 for the period before and following 9/11 (compared to similar data from other years). We hope to determine if the observed reduction in vessel activity post-9/11 resulted in a decline in ambient noise, and if there was an accompanying decrease in stress hormones in the right whales inhabiting the Bay after this event.

APPROACH

The approach in FY 2011 was to: 1) complete health assessments, 2) develop models of the relationships between fecal stress and reproductive hormone levels to characterize (and control for) variations in stress hormones that occur with different sexes, ages and reproductive states; 3) model the links between Body and Skin Condition scores and fecal stress hormones; 4) complete analyses and write up the hormone and acoustic data collected before and after 9/11 and in the surrounding years to compare the effects of ambient noise levels on whale physiology, and 5) upgrade our endocrine laboratory capabilities to conduct retrospective assays, and develop new hormone assay techniques. Our team includes Scott Kraus, New England Aquarium (right whale biology), Roz Rolland, New England Aquarium (mammalian physiology, health assessments), Peter Corkeron, National Marine Fisheries Service (statistics), Kathleen Hunt (endocrinology) and Susan Parks, The Pennsylvania State University (acoustics).

WORK COMPLETED

Task 1a: Health Assessments

The visual health assessment database is currently being updated for all right whale sightings between 2008 and 2009. Details of the visual health assessment method and scoring criteria can be found in

Pettis et al. (2004). The North Atlantic Right Whale Catalog and Database holds records for a total of 13,916 sightings of right whales between December 2007 and December 2009. At the time of this report, 11,786 of these sightings have been grouped by habitat and year into 1,444 batches. Of these, 501 batches have been evaluated and scored for the four visual health parameters (Table 1). The updated Health Assessment Database now consists of a total of 11,145 batches and 40,794 sightings.

Table 1. Total number of batches and sightings evaluated and assessed for visual health assessment database update, 2008-2009.

Year	Batch Count	Sighting Count
2008	256	653
2009	245	791
Total	**501**	**1,444**

Task 1b: Linking Hormones and Health Assessments

Determining relatively stressed animals using conditional inference trees.

Previous work at the New England Aquarium has demonstrated that immunoassay of whale feces provides biologically relevant information on levels of reproductive and “stress” (glucocorticoid, GC) hormones (Rolland et al. 2005, Hunt et al. 2006). Here, we used conditional inference trees (CIT’s), which take a statistical approach to partitioning, accounting for the distributional properties of the data (Hothorn et al. 2006). Data used for this analysis were from fecal samples from identified whales of known gender and reproductive state. Reproductive state was the classifying variable, and the four hormones (androgens, progesterone, estrogen and glucocorticoid hormones) were the independent variables. Calves, fetuses and juveniles of unknown gender were not included, leaving 95 samples for analysis.

Using CIT classification of both reproductive and stress hormone samples directly assigned to individual whales of known reproductive state, all pregnant females, most (22 of 23) mature males, most (20 of 23) lactating females, and most (19 of 20) juvenile females were correctly assigned. Only resting females (no class determined, so all 8 were misclassified) and juvenile males (5 of 14 correctly assigned) were problematic, although just over half of the “juvenile” males assigned to “mature” males (4 of 7) could have been mature or maturing (age unknown but at least 6 or 8 yrs, and known minimum age of 8). One CIT node included 8 animals with relatively high levels of cortisol, given their other hormone levels. Of these, two were females nursing their first calf, a mature male incorrectly classified due to low androgen levels, a resting female killed by ship strike, an immature animal that was infected with *Giardia* and *Cryptosporidium*, a resting female in the middle of a six-year calving interval (indicating the possibility of a lost calf), and an apparently healthy juvenile. These results suggest that hormones sampled from feces provide a reliable indicator of physiologic stress in right whales.

Body condition indices and stress levels.

Next, to determine if there is a relationship between right whales' levels of stress (glucocorticoid) hormones and body condition, we used known animals with fecal hormone data and concurrent health assessment scores. Eighty samples were used in this analysis. For most health assessment indices there were few examples of whales with scores other than those indicative of the best health. The exception

to this was the Body Condition Index, so all further analyses were conducted on those data. This left insufficient samples to construct conditional inference trees, requiring a more classical statistical approach.

To analyze the relationship between fecal GC levels and Body Condition Scores, we ran a nested linear model, initially with GC level as the dependent variable, and the other hormone levels nested in reproductive state as the explanatory variables. As there were no instances of pregnant females with a body condition score other than 1, and only 1 resting female in the data available for analysis, they were removed. This left 64 samples for the analysis. A nested linear model was taken as a base model, to which body condition score was added (as a factor), also nested by reproductive state. The change in AIC between the base model and the model including body condition showed that including body condition improved the fit of the model to the data (Table 2). Comparing deviances between the models with an F-test (Venables and Ripley 2002) confirmed this.

Table 2. Analysis of Deviance Table: Model 1: base, nested model: Model 2: Body Condition Code (nested) added to the model.

	Residual Df	Residual Deviance	Df	Deviance	F	Pr(>F)
1	44	23746				
2	40	15828	4	7918.9	5.0033	0.002296

In addition, mature males with body condition greater than 1 (thin and emaciated) had significantly higher GC levels than mature males with body condition=1 (fat) ($t=4.421$, $p<0.001$).

Task 1c. Health Assessment Indices and Life History

Body Condition Effects on Reproduction

To determine the effects of body condition on reproduction, we analyzed the relationship between the Body Condition Index and calving in adult females for the period 1980-2007. The change in state of individual adult females was treated as a Markov chain process, which quantifies the way in which an event depends on the event that immediately precedes it, and provides a way to calculate the probabilities of one event transitioning to another. This method is used to construct population projection matrices for animal populations (Caswell 2001). However, in this analysis we constructed individual projection matrices, tracking the probability of each adult female right whale transitioning between three possible events – Pregnancy, Lactation and Resting. Female right whales usually have a three-year calving cycle, in which they are pregnant in one year, nurse a calf the next, and rest in the third year.

Only female whales with at least 20 years of sightings as an adult were used in analysis (n=49). None of these individuals were ever recorded as having a Body Condition of 3 (emaciated). For each individual whale, a transition matrix was constructed, showing the state that each whale was in each year, along with the stage that the individual moved to in the following year. The popbio library (Stubben and Milligen 2007) was used to construct a projection matrix model from each individual's transition matrix and, and the plyr library (Wickham 2011) was used to simplify the code to process the construction of all 49 matrices. An example of a projection matrix (for right whale 1004) is:

	From:		
To:	**preg**	**lact**	**rest**
Preg	0	0	0.2857143
lact	1	0	0.0000000
rest	0	1	0.7142857

In this simplified matrix, fecundity (as classically calculated in population projection matrices) and body condition categories (e.g. preg1, preg2, preg?) have been excluded. This matrix follows presentation standards in population biology (Caswell 2001), with "from" in columns, and "to" in rows. So in this instance, whale 1004 always went from pregnant to lactating (column 1, row 2), and from lactating to resting (column 2, row 3), from resting to pregnant with a probability of about 0.29 (column 3, row 1), and remaining resting with a probability of about 0.71 (column 3, row 3).

In analyses for all 49 whales, females almost never transitioned from resting with a body condition of 2 (R2, i.e. in relatively poor condition) to pregnant. Although unsurprising – this normal mammalian behavior, especially for capital breeders like baleen whales – this initial analysis demonstrates that poor Body Condition is associated with reduced reproduction.

Body Condition Effects on Mortality

Because most deaths in right whales are not usually detected, the Right Whale Catalog assumes that the disappearance of whales for 6 years indicates the whale has died. This assumption appears to be correct over 95% of the time. However, some whales are re-sighted after longer intervals – these whales are classified as "resurrected". If body condition influences the likelihood of survival, then the whales that are presumed dead due to a gap in sightings and never seen again (i.e., those that probably died) should have worse body condition in their last sighting than those whales that are presumed dead but are later re-sighted (gap of sightings > 6 years).

From the health assessment database, all whale Body Condition Scores were extracted for each whale's last sighting before their disappearance. Table 3 shows the whale data by Body Condition and mortality status (presumed dead and resurrected vs presumed dead and never seen again). A Chi-squared analysis shows that those whales coded with a Body Condition 3 at last sighting were significantly more likely to die than those with better body conditions (Table 4). In addition, removing the Body Condition 3 whales from this analysis showed that whales in Body Condition 2 were also significantly more likely to die than whales in Body Condition 1 ($p = 0.036$).

Table 3. Right whale body condition and mortality status

Status	Body Condition			Total
(BC = Body Condition Code)	Normal (BC=1)	Thin (BC=2)	Emaciated (BC=3)	
Presumed Dead/Resurrected	30	5	0	35
Presumed Dead/Never seen again	61	30	9	100
Total	91	35	9	135

Table 4. Chi-squared analysis of data in Table 3.

Chi-Square Tests			
	Value	Df	Asymp. Sig. (2 sided)
Pearson χ^2	7.969[a]	2	0.019
Likelihood Ratio	10.43	2	0.005
n of valid cases	135		
Note a. 1 cell (16.7%) has an expected count less than 5. The minimum expected count is 2.33.			

Task 2: The 9/11 analysis
The events of 11 September 2001 (9/11) resulted in an unanticipated experiment on the effects of ambient noise on western North Atlantic right whales (*Eubalaena glacialis*) in the Bay of Fundy, Canada. We compared the sound pressure level (SPL) and frequency spectrum from recordings on two days before 9/11 with two days immediately following 9/11. Acoustic data showed a 6dB decrease in underwater noise below 150 Hz related to reduced ship traffic following 9/11. In a concurrent study, we compared levels of glucocorticoid metabolites (GCs; "stress hormones") measured by radioimmunoassay in right whale fecal samples before (n =114) and after 11 September (n = 30) for the years 2001-2005. The only year in which there was a significant decrease in fecal GCs after 11 September was 2001 with a significant effect of year and period (Kruskall-Wallis X^2 = 29.6889, df = 4, $P < 0.0001$) (Figure 2). Consistent surveys throughout the study period revealed no other factors that could explain this difference besides the decrease in ship traffic and reduced underwater noise following 9/11. This is the first evidence that exposure to underwater noise from large ships is associated with a measure of chronic stress in free-ranging whales, which has implications for baleen whale populations in heavy ship traffic areas.

The full paper is currently in review at a peer-reviewed journal with the following title and authorship. *Reduced Ship Noise Lowers Stress Hormones in Right Whales,* by Rosalind M. Rolland, Susan E. Parks, Kathleen E. Hunt, Manuel Castellote, Peter J. Corkeron, Douglas P. Nowacek, Samuel K. Wasser and Scott D. Kraus.

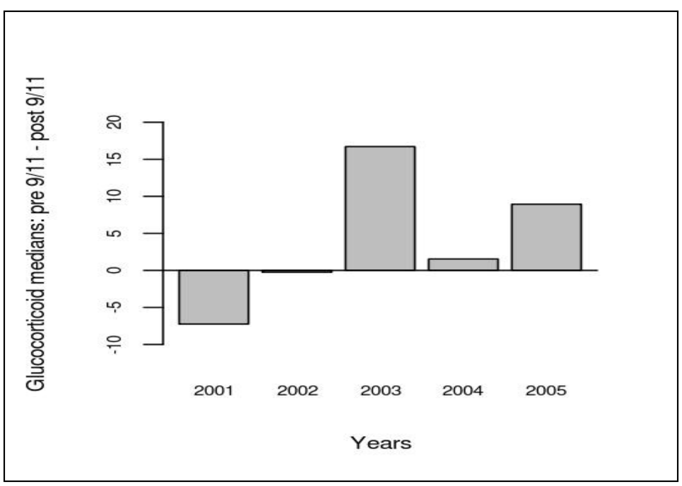

Figure 2. Change in median fecal glucocorticoid levels from right whales before and after 11 September in the years 2001-2005.

Task 3: Lab upgrades, hormone analyses, validation of thyroid hormones.
Lab renovations were delayed by unexpected discoveries of HVAC, fire suppression, and electric problems during construction. However, the core renovation was completed in early June, lab equipment was progressively installed and tested in sequence during June-September 2011, and the lab is considered fully operational. The Labconco lyophilizer has been on-line and drying samples since June 2011. Equipment for corticosterone and estrogen assays (gamma counter, etc.) came on-line in July 2011, and both these assays are fully operational. Equipment for progesterone and testosterone assays (scintillation counter, etc.) came online in August 2011, and both these assays are now in the final stages of testing. Due to delays in compressed-air hookup in the lab, the last assay to come online has been the thyroid assay; we began testing this assay in late September 2011. Despite the delays in lab construction, more than half of the planned PCAD labwork is completed. All archived right whale fecal samples from the last half of 2005 through 2010 have been completely prepared for assay (freeze-dried, sifted, weighed, extracted, and diluted in appropriate buffers). All corticosterone assays were completed in August 2011, and estrogen assays are 50% completed. The progesterone and testosterone assays are scheduled for October 2011. We have successfully completed the first thyroid assay validation, which indicated that right whale feces do contain detectable thyroid hormone. Remaining validations for this assay are scheduled for October 2011, and final assays for November 2011. The completion of the lab assays will coincide with the completion of the remaining health assessments near the end of the year. All updated information from these two data streams will be incorporated into the revised matrix models in the winter of 2012.

RESULTS

To date we have demonstrated that 1) fecal GC levels reflect physiologic stress in right whales, (2) higher GC levels are linked with poorer body condition, (3) poor Body Condition Scores are associated with reduced female reproductive output and increased likelihood of mortality, and (4) in the 9/11 analysis, lower GC levels in right whales were associated with reduced low frequency noise from shipping. We have not attempted to model causation in this approach, but combined, these findings show strong evidence of linkages between marine noise, stress, body condition (health), and both reproduction and mortality.

IMPACT/APPLICATIONS

The successful use of fecal stress hormones, visual health assessments, and appropriate statistical methods suggest these tools in the appropriate experimental, field, and/or comparative setting, can determine the relationships between anthropogenic (or other) stressors and their effects on whales.

RELATED PROJECTS

As part of the New England Aquarium's Marine Health Program, we have expanded the stress hormone analytical approach to include fecal hormones in beaked and sperm whales (Roz Rolland, PI; ONR Contract # N000141110540), as well as preliminary studies on the detection and use of hormone data from right whale respiratory exudate (Kathleen Hunt, PI; ONR Contract # N000141110435) .

REFERENCES

Caswell, H, 2001. Matrix population models. Sinauer.

Hothorn, T., Hornik, K., & Zeileis. A. 2006. Unbiased Recursive Partitioning. Journal of Computational and Graphical Statistics. 15(3): 651-674.

Hunt KE, Rolland RM, Kraus SD, Wasser SK. 2006. Analysis of fecal glucocorticoids in the North Atlantic right whale (*Eubalaena glacialis*). General and Comparative Endocrinology 148:260-272.

National Research Council of the U.S. National Academies (NRC). 2005. Marine Mammal Populations and Ocean Noise: Determining When Ocean Noise Causes Biologically Significant Effects. National Academy Press, Washington, DC.

Pettis HM, Rolland RM, Hamilton PK, Brault S, Knowlton AR, Kraus SD. 2004. Visual health assessment of endangered North Atlantic right whales (*Eubalaena glacialis*) using photographs. Canadian Journal of Zoology 82:8-19.

Rolland RM, Hunt KE, Kraus SD, Wasser SK. 2005. Assessing reproductive status of right whales (*Eubalaena glacialis*) using fecal hormone metabolites. General and Comparative Endocrinology 142:308-317.

Stubben, C.J. and Milligan, B.G. 2007. Estimating and Analyzing Demographic Models Using the popbio Package in R. Journal of Satistical Software 22:11.

Venables, W.N., & Ripley, B.D. 2002. Modern applied statistics with S. New York, Springer, 4th ed.

Wickham, H. (2011). The Split-Apply-Combine Strategy for Data Analysis. Journal of Statistical Software, 40(1), 1-29. URL http://www.jstatsoft.org/v40/i01/. Springer NewYork.